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# Through a glass darkly: salt transport by the distal tubule

David H. Ellison[1,2]

**The distal convoluted tubule (DCT) plays a central role in blood pressure and potassium homeostasis, as evidenced by diseases that occur when its function is modified. The paper by van der Lubbe and colleagues makes clear that angiotensin II itself increases the activity and abundance of the thiazide-sensitive Na-Cl cotransporter (NCC), independent of changes in circulating aldosterone. This Commentary provides additional perspective on that work.**

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"*For now we see through a glass, darkly; but then face to face.*"

–1 Corinthians 13

These are exciting times for the distal convoluted tubule (DCT), or at least for those who study it. During the golden age of micropuncture, this short nephron segment was studied widely. Later, however, attention shifted to other nephron segments, owing to ease of study and the belief that NaCl transport along the DCT is determined 'in large part by delivered load,' with only 'equivocal' evidence for regulatory modulation.[1] New molecular tools and techniques, coupled with exciting insights into genetic hypertension and salt wasting, however, now identify the DCT as a key site for regulated NaCl transport. As with any field that is moving rapidly, however, emerging results often raise confusing questions. Our understanding of DCT transport remains inchoate, but the paper by van der Lubbe and colleagues[2] (this issue) helps to bring some clarity.

During the past 15 years, evidence has accumulated that aldosterone increases sodium reabsorption along the DCT.[3] More recently, arginine vasopressin (AVP) has also been shown to enhance sodium reabsorption along this segment.[4] Aldosterone and AVP have long been known to stimulate Na transport along the cortical collecting duct, by acting on the epithelial Na channel (ENaC); AVP also increases water permeability of this segment (via aquaporin-2), where both the mineralocorticoid receptor (MR) and the vasopressin type 2 receptor (V2R) are expressed. Yet DCT cells also express MR[5] and V2R.[6] These receptors probably mediate direct hormonal effects in the DCT, as aldosterone increases the activity[3] and abundance[7] of the thiazide-sensitive Na-Cl cotransporter (NCC), as does AVP.[4,8,9]

The dominant NaCl transport pathway of the DCT is NCC. To transport NaCl, NCC must move ('traffic') to, and be inserted into, the apical plasma membrane; it is also phosphorylated along its amino-terminal cytoplasmic domain, enhancing activity (Figure 1). WNKs are intracellular kinases that modulate NCC activity by altering both trafficking and phosphorylation. WNK4 reduces NCC movement to the apical membrane[10] from sites where it is synthesized (the endoplasmic reticulum) and processed (the Golgi apparatus), at least in part, by targeting it to lysosomes, where it can be degraded;[11,12] the effects of WNK4 may be modulated by angiotensin II (see below). In contrast, WNK3 increases NCC abundance and activity.[13–15] Thus,

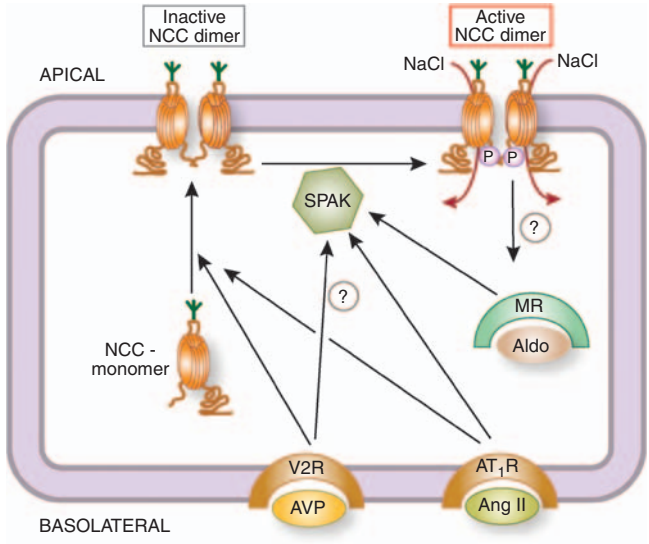


**Figure 1 | Simplified scheme of regulation of thiazide-sensitive Na-Cl cotransporter.** The thiazide-sensitive Na-Cl cotransporter (NCC) is synthesized and then glycosylated (green forks) within the Golgi apparatus (not shown, for clarity). NCC then moves to and into the apical membrane, where it exists as a dimer. To be fully active, NCC undergoes phosphorylation along its amino-terminal cytoplasmic domain, mediated largely by SPAK, thereby permitting NaCl transport. Little is known about mechanisms of removal from the membrane. Arginine vasopressin (AVP), aldosterone (Aldo), and angiotensin II (Ang II) all stimulate NCC activity. Trafficking may be a rapid effect, modulated predominantly by AVP and Ang II. Phosphorylation may occur within the membrane and is enhanced by all three factors.

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some WNKs are predominantly inhibitory, whereas others are predominantly stimulatory, at least with respect to NCC. Little is known about how NCC is removed from the apical membrane, although the process does not appear to involve clathrin-mediated endocytosis.[12,16]

As noted above, NCC is also activated by phosphorylation (Figure 1). Phosphorylation activates NCC without changing its membrane abundance, at least when it is expressed heterologously in *Xenopus* oocytes.[17] The major kinase that phosphorylates and activates NCC appears to be SPAK.[18,19] SPAK, which is expressed along the distal nephron,[20] can itself be phosphorylated and activated by WNK kinases, so that WNK, SPAK, and NCC constitute a signaling pathway.[21] Nevertheless, although kinase domains of the several WNKs are homologous, all WNKs do not appear to have the same effects on NCC. As noted above, WNK4 appears to act as an inhibitor of NCC,[10,22] at least under some conditions,[23] whereas WNK1 phosphorylates SPAK to activate NCC.[19] In HeLa cells, WNK1, but not WNK4, activated SPAK and caused a large shift in electrophoretic mobility;[24] thus, details of how WNK kinases modulate NCC remain confusing.

Angiotensin II is another component of the renin–angiotensin–aldosterone system that stimulates Na transport along the DCT.[25] This effect is also likely to be direct, owing to the presence of angiotensin II type 1 ($AT_1$) receptors along the DCT.[26] Genetic deletion of $AT_{1a}$ receptors reduces the abundance of NCC,[27] and infusion of angiotensin II for 8 days increases the abundance and phosphorylation of NCC;[28] thus, angiotensin II and aldosterone appear to have similar effects on NCC activity. Gamba and colleagues reported that angiotensin II relieved the inhibitory effect of WNK4 on NCC, in a SPAK-dependent manner.[23]

Angiotensin II increases NCC activity, in part, by increasing the abundance of NCC at the apical plasma membrane. This effect occurs rapidly, with short-term angiotensin II infusions increasing the ratio of apical to subapical NCC.[29] In cultured mpkDCT cells, angiotensin II also increases SPAK and NCC phosphorylation, suggesting that acute exposure to angiotensin II also activates the transporter allosterically.[28] Longer-term effects, induced by dietary NaCl restriction[30] or angiotensin II infusions,[28] also stimulate the activity of NCC and increase its abundance and its phosphorylation; in these situations, however, the effects may be direct, from $AT_1$-receptor activation, or indirect, via aldosterone stimulation.

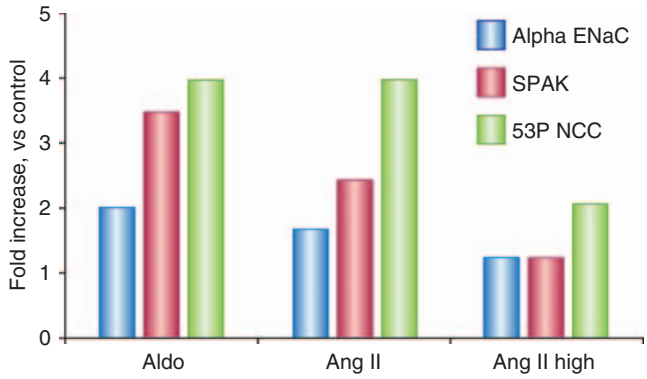


**Figure 2 | The effects of aldosterone, angiotensin II, and pressor-dose angiotensin II on abundance of αENaC, SPAK, and phosphorylated NCC.** Note that all three interventions increase phosphorylated NCC, whereas aldosterone increases the abundance of the α-subunit of the epithelial Na channel (αENaC) mimimally (Ang II) or not at all (Ang II high). Please see text for more details. Aldo, aldosterone; Ang II, angiotensin II; Ang II high, pressor-dose angiotensin II. (Based on the data of van der Lubbe *et al.*[2])

Talati and colleagues concluded, on the basis of inhibitor studies, that long-term effects of angiotensin II on NCC are mediated by aldosterone[28] and suggested therefore that aldosterone is the predominant NCC regulatory factor. The paper by van der Lubbe and colleagues[2] (this issue) shows clearly that angiotensin II itself increases NCC abundance and phosphorylation, even during chronic exposure; the authors used the definitive approach of performing adrenalectomy, and then infusing hormones chronically, to fix adrenal steroid concentrations. The results are clear: angiotensin II increases NCC abundance and phosphorylation even when serum aldosterone levels are fixed. Several additional points, derived from their data, however, deserve emphasis.

First, Figure 2, based on the data of van der Lubbe,[2] shows that aldosterone, but not angiotensin II, substantially increased the abundance of the α-subunit of ENaC (αENaC). This pattern of hormonal effect on ENaC contrasts with effects on NCC, in which both angiotensin II and aldosterone increase NCC abundance and phosphorylation. These results help to explain how aldosterone, a single hormone, can generate either NaCl retention or Na/K exchange, depending on the stimulatory signal (an effect termed the 'aldosterone paradox'[31]). Thus, when aldosterone secretion is stimulated by angiotensin II (such as occurs when the extracellular fluid volume is depleted), Na reabsorption will be stimulated along much of the nephron (including the proximal and distal tubule, by angiotensin II, and the distal tubule and collecting duct, by aldosterone). These effects will restore extracellular fluid volume both because proximal segments reabsorb NaCl, and because Na delivery to the distal, K secretory sites will be limited. In contrast, when aldosterone secretion is stimulated by hyperkalemia, in the absence of changes in angiotensin II, Na reabsorption will only be stimulated distally, favoring the exchange of Na for K. Although other mechanisms are likely to be involved, the patterns of angiotensin II and aldosterone effect on Na transport along the nephron certainly reflect physiologically adaptive processes.

Second, while NCC stimulation by either angiotensin II or aldosterone is associated with increases in SPAK abundance and SPAK phosphorylation, when animals received higher doses of angiotensin II, NCC appeared to be stimulated, even though SPAK (and phosphorylated SPAK) were at baseline levels; even though this effect did not quite reach statistical significance, it raises the possibility that other kinases can activate NCC.

Finally, although comparisons of protein abundance do not necessarily reflect changes in transporter activity, the ability of aldosterone to increase NCC abundance is quite impressive, in comparison with its ability to increase ENaC abundance. Many, if not most, introductory texts for medical and graduate students describe effects of aldosterone on ENaC but omit effects on NCC.[32] The accumulating data suggest that it is time to break old paradigms, and include NCC as a crucial aldosterone-regulated transport protein, when introducing students to the effects of adrenal steroids on the kidney.

Lest the current data be seen as clearing all confusion, several questions remain. As noted above, two groups[8,9] have shown that AVP increases trafficking and phosphorylation of NCC. In the study by van der Lubbe and colleagues,[2] the abundance of aquaporin-2 was increased by both aldosterone and angiotensin II infusion. This suggests either that these peptides stimulated AVP secretion or that angiotensin II activated V2R directly; there is some evidence in support of the second model.[33] From a physiological standpoint, of course, the striking similarity of effects of aldosterone and AVP on distal transporters is hard to reconcile with effects on whole-animal balance. Aldosterone and AVP both stimulate ENaC *and* NCC. Yet hyperaldosteronism typically presents with hypertension, owing to sodium chloride retention, while the syndrome of inappropriate antidiuretic hormone secretion presents with hyponatremia, owing to effects on aquaporin-2, but without NaCl retention. This suggests either that the potency of stimulatory effects on Na transport, or the escape mechanisms that supervene, are different, or that other factors come into play. One possible factor is V1a receptors; most studies of AVP actions use the V2-receptor-specific agonist desmopressin (dDAVP). V1a receptors, a second target of the native hormone AVP, can increase natriuresis.[34]

Finally, the roles played by WNK kinases in modulating or mediating effects of angiotensin II and/or aldosterone remain intriguing but are not fully elucidated. In view of the phenotype that results when WNK kinases are mutated, familial hyperkalemic hypertension (pseudohypoaldosteronism type II or Gordon syndrome), it seems clear that these kinases help to determine whether aldosterone is primarily kaliuretic or NaCl retentive. Yet changes in WNK4 were not observed in the experiments reported by van der Lubbe and colleagues,[2] and data concerning WNK1 or WNK3 are not reported. WNK kinases may play a crucial role in determining NCC membrane abundance and states of phosphorylation, but the roles of the individual players, and their integration, remain poorly understood. Further, it seems likely that effects of WNK kinases, or at least WNK4, are modulated by circulating (or local) levels of angiotensin II,[23] as noted above. Much remains to be learned about the interactions between WNKs, SPAK, NCC, and the rennin-angiotensin-aldosterone system. Yet the possibility that small-molecule WNK modulators might provide novel ways to 'turn down' the distal nephron means that this pathway is an attractive target for drug development.

## DISCLOSURE

The author declared no competing interests.

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# Kidney function for the non-nephrologist: an emerging tool for predicting mortality risk

Stein I. Hallan[1]

**Estimated glomerular filtration rate (eGFR) and albuminuria are among the most important cardiovascular risk factors, but the optimal cutoff for predicting mortality may not yet have been agreed upon. Foley *et al.* analyzed data from the population-based NHANES III study with classification tree methodology. They found that an eGFR of 94 ml/min per 1.73 $m^2$ and an albumin–creatinine ratio of 9 mg/g were the optimal cutoff values, that is, more 'normal' values than are used to define chronic kidney disease.**

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Cardiovascular disease has been a major public-health problem for more than 50 years in the developed world. Enormous research efforts have been undertaken to understand cardiovascular diseases, and great progress has been made in both prevention and treatment. During the past 20 years, we have experienced a tremendous decline in cardiovascular mortality among middle-aged people, and substantial improvements in treatment have also been achieved in the elderly.[1]

Discovering major risk factors for cardiovascular diseases and organizing these into risk prediction scores have been important in this progress. In the INTERHEART study, nine risk factors accounted for 90% of the population attributable risk for incident myocardial infarctions.[2] However, this might be too optimistic, and, at least in clinical practice, we are not able to predict 90% of all future cardiovascular events. The 'number needed to treat' to prevent one cardiovascular event is still often estimated to be around 100.[3] This rather high number is probably due to both lack of treatment effectiveness and suboptimal risk stratification of the patients, and, especially in the elderly, the latter could be a substantial problem. Both the Framingham risk equation and the Systematic Coronary Risk Evaluation (SCORE) equation were developed for subjects younger than 70 years old. Likewise, the predictive value of major risk factors such as hypertension and dyslipidemia is reduced in the elderly.[4] The reasons for this 'reversed epidemiology' are several, but survival bias is probably one of the most important. To improve risk stratification testing for subclinical atherosclerosis could be one possible way forward, especially in the elderly. Measurement of carotid intima–media thickness and measurement of coronary calcifications with ultrasound and computed tomographic scanning, respectively, are techniques with high diagnostic accuracy but low availability.[5] Chronic kidney disease has been increasingly suggested as a potential 'test' for cardiovascular risk prediction, as it has high diagnostic accuracy like the aforementioned methods, has no side effects, and is much cheaper. However, the optimal use of estimated glomerular filtration rate (eGFR) and urinary albumin excretion is not yet agreed upon.

Foley *et al.*[6] (this issue) make an interesting contribution to this topic. Using the large population-based Third National Health and Nutrition Examination Survey (NHANES III) cohort from the United States, they studied how well kidney function and other risk factors predicted mortality risk, using classification tree analysis. Their main finding is that, for optimal prediction of mortality risk, more 'near-normal' cutoff values for kidney function should be used. The current 2002 Kidney Disease Outcomes Quality Initiative (KDOQI) uses an eGFR below 60 ml/min per 1.73 $m^2$ and an albumin–creatinine ratio above 30 mg/g for definition and risk classification of chronic kidney disease and its outcomes. Cutoffs for mortality-risk prediction could, however, be different, and Foley *et al.*[6] suggest using an eGFR of 94 ml/min per 1.73 $m^2$ and an albumin–creatinine ratio of 9 mg/g as

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